IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Trinkle *et al*
Application No. 10/509,570
Filed: September 29, 2004
Confirmation No. 8359
For: CHITOSAN PRODUCTION
Examiner: Layla D. Bland
Art Unit: 1623
Attorney Reference No. 6682-77985-03

FILED VIA EFS **January 31, 2008**

COMMISSIONER FOR PATENTS
P.O. BOX 1450
ALEXANDRIA, VA 22313-1450

DECLARATION OF JAMES TRINKLE

1. I, James Trinkle, am a co-inventor of the above-referenced patent application. I have over 20 years of experience in industrial production of citric acid and by-products thereof, such as chitosan and glucosamine. In addition to my work experience I hold a degree in Biochemistry from Iowa State University.

2. I understand that the U.S. Patent Office has rejected the claims that are presently pending in the above-referenced application based upon the Hu *et al.* reference, Butelman, *et al.* reference, and the Konda *et al.* reference. I also understand that the Patent Office has found the data presented in the above-referenced application to not be clear and convincing. Accordingly, I will explain herein the data and provide a further explanation of the differences between the cited references and the claimed subject matter.

3. Chitin exists in fungal cell walls as a matrix in which the chitin is bound to glucans by hydrogen bonds in a complex helical structure (see, page 125 of Kogan, *Studies in Natural Products Chemistry*, Volume 23, Elsevier Science B.V. pages 107-152, 2000, copy enclosed). During the production of chitosan from fungal cell walls, chitin is deacetylated and depolymerized at different rates. The rates of depolymerization and

deacetylation are impacted by the availability of the chitin within the chitin/glucan complex, as well as the depolymerization rate of the glucans themselves.

4. Glucans and their breakdown products present significant hurdles in the industrial processing of fungal biomass to chitosan. Glucans impact the ability to produce chitosan having desired molecular weights and deacetylation levels, and the separation of glucans adds significant recovery and production costs. Hence, a simple, one-step process for producing chitosan from a fungal biomass that is relatively free of glucans is desirable.

5. In order for chitosan to become detectable using an acid-solubilization based assay (examples of such assays are provided in Hu *et al.*, as well as the above-referenced application) the chitosan must obtain a minimal level of deacetylation. For example, a chitosan polymer that has a molecular weight of about 100,000 with about 50,000 units (50%) deacetylated will be soluble in acid and be detectable; however, a chitosan molecule that has a molecular weight of about 250,000 with about 50,000 units (25%) deacetylated will not be detectable. During a caustic based chitosan production method depolymerization will also occur. Hence, at the beginning of a production process, prior to a significant amount of depolymerization occurring, initially only shorter chitosan polymers will be detected. Then as the chitin/glucan complex is broken down, exposure and deacetylation occurs and the larger polymers become detectable. Eventually, as the reaction continues, all the chitin is converted to chitosan, but depolymerization will continue reducing the chitosan polymer molecular weight. The discovery of the complexity of these reaction dynamics is what inspired the above-referenced application.

6. Table 1 in the above-referenced application provides an illustration of the information described in paragraph 5, above. Initially, upon 4 hours of exposure to 20.1% NaOH the average size of the chitosan molecules is relatively shorter and on average 79% deacetylated. However, at 12 hours the average size of the chitosan molecules being released from the glucans is larger and 82% deacetylated. At 16 hours the impact of

the depolymerization of the chitosan starts to play a prominent role in the reaction and the average molecular weight drops off.

7. Turning to the Hu *et al* reference, Hu *et al* concludes that time of treatment is a crucial factor in degradation and that longer treatment times produced chitosan with the lowest molecular weight ($7.84x10^4$) (see page 192, second full paragraph). Given this conclusion one of ordinary skill in the art, such as myself, would not have concluded that the time of treatment should be increased to produce higher average molecular weight chitosan. Additionally, because arthropods (which were used in the Butelman *et al.* reference) do not have chitin/glucan complexes the caustic can more directly act upon the chitin and therefore, there is a more linear relationship between time of caustic treatment and average molecular weight. Hence, the conclusions or results provided in the Butelman, *et al.* reference cannot be transferred to methods of producing chitosan from fungal biomass.

8. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. § 1001, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

JAN. 30, 2008
Date

James R. Trinkle
James Trinkle